AvidiMab®, an avidity-enhancing platform for cancer immunotherapy

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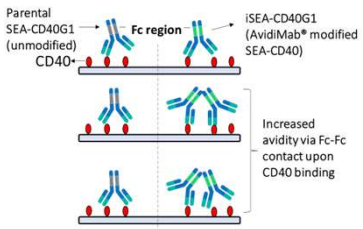
INTRODUCTION

- **Avidity, the combined binding strength of individual interactions, is a key aspect of cancer-targeting by therapeutic antibodies (mAbs)**
- **AvidiMab®-engineered anti-glycan mAbs have improved *in vitro* and *in vivo* anti-tumour activity [1]**
- **Fc-engineering AvidiMab® technology is proposed to enhance non-covalent Fc:Fc associations by neighbouring target-bound mAbs [2]**
- **CD40 agonists are promising immune therapeutics mimicking CD40L action by crosslinking CD40, thereby improving antigen presentation and expanding tumor-specific cytotoxic T cells [3]**
- **SEA-CD40 is a clinical-stage CD40 agonistic mAb. Fc-engineered AvidiMab® SEA-CD40, 'iSEA-CD40G1' was created in IgG1 format and the impact of enhanced avidity evaluated using a range of techniques and in cell-based assays**

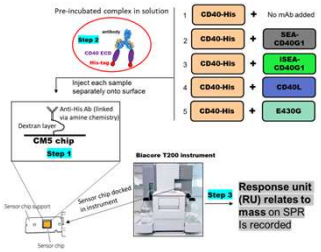
Fc-engineering AvidiMab® technology

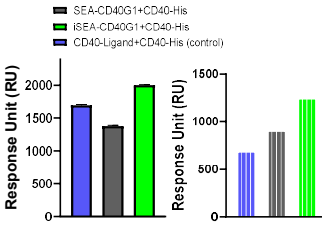


- Fc engineering of human SEA-CD40 IgG1 (AvidiMab® technology) produced iSEA-CD40G1. CD40 binding by iSEA-CD40G1 promotes self-association thereby increasing avidity and functionality

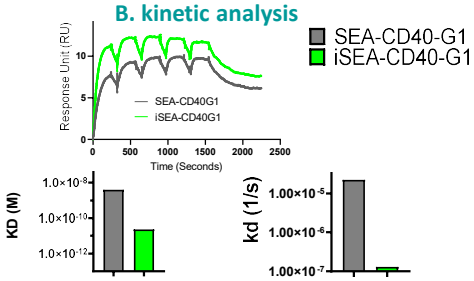
improved CD40 binding by AvidiMab®-engineered iSEA-CD40G1

A. binding capacity





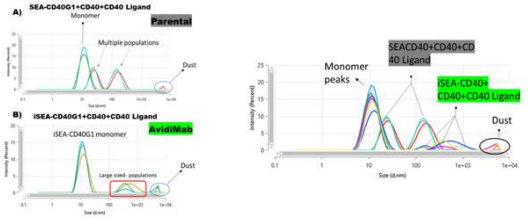
B. kinetic analysis



A. SPR binding analysis - higher Response Units (RU) captured by CD40-His pre-incubated with iSEA-CD40G1 compared to parental SEA-CD40G1 (left panel), suggesting higher-order complex formation due to Fc:Fc association. The same trend was seen when the antibody complex formation was performed with preformed CD40:CD40 Ligand (right panel)

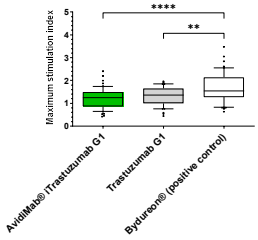
B. SPR kinetic analysis (single-cycle) - higher avidity (lower KD) and slower off-rate (k_d) for AvidiMab® iSEA-CD40G1 binding to captured CD40 (His-tagged) compared to parental SEA-CD40G1

higher order complex formation by AvidiMab® iSEA-CD40G1+CD40+CD40 Ligand



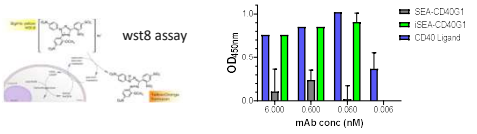
- size distributions (dynamic light scattering, DLS) for pre-incubated CD40 + CD40 ligand in the presence of parental SEA-CD40G1 compared to AvidiMab® iSEA-CD40G1
- in addition to antibody monomer peaks, larger complex formation peaks are observed for AvidiMab® iSEA-CD40G1 compared to parental SEA-CD40G1, indicating more efficient CD40 clustering

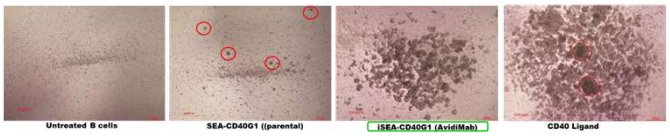
AvidiMab® engineering is non-immunogenic



- similar frequency (6%) and maximum stimulation (SI) of proliferative responses by iTrastuzumab and Trastuzumab (Herceptin®) in a 50-donor cohort
- significantly lower proliferative responses compared to the positive control Bydureon®

enhanced B cell activation and proliferation by AvidiMab® iSEA-CD40G1





- top: enhanced B cell proliferation in the presence of AvidiMab® iSEA-CD40G1 compared to parental SEA-CD40G1; CD40 Ligand included as a control
- bottom: enhanced B cell clustering as a qualitative readout of B cell activation/proliferation in the presence of AvidiMab® iSEA-CD40G1 compared to parental SEA-CD40G1; CD40 Ligand included as a control

CONCLUSIONS

- **iSEA-CD40G1 (Fc-engineered AvidiMab® technology) displayed improved CD40 functional affinity/avidity compared to parental SEA-CD40G1**
- **iSEA-CD40G1 induced superior B cell proliferation compared to SEA-CD40**
- **AvidiMab® Fc-engineering carries a low immunogenicity risk, similar to Herceptin®**
- **enhanced target-driven clustering through AvidiMab® Fc-engineering increases avidity - independent of Fc gamma receptors - for superior functionality**
- **broader applicability of the AvidiMab® technology is actively being explored**

References

1. Vankemmelbeke M, McIntosh RS, Chua JX, Kirk T, Daniels I, Patsalidou M, et al. Engineering the Human Fc Region Enables Direct Cell Killing by Cancer Glycan-Targeting Antibodies without the Need for Immune Effector Cells or Complement. Cancer Res. 2020;80(16):3399-412
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3. Salomon R, Dahan R. Next Generation CD40 Agonistic Antibodies for Cancer Immunotherapy. Front Immunol. 2022;13:940674